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In Vitro and *in Vivo* Inhibition of Human Papillomavirus Type 16 *E6* and *E7* Genes

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ABSTRACT

Human cervical cancers are often associated with human papillomavirus (HPV). In HPV-positive cervical cancers, the oncoproteins E6 and E7 are consistently expressed. In this study, the effects of antisense inhibition of both proteins were examined. Phosphorothioate oligonucleotides (ODNs) AE6 and AE7 complementary to regions flanking the start codons of HPV16 *E6* and *E7* genes, respectively, were synthesized. These anti-HPV ODNs inhibited the growth of cervical cell lines CaSki and SiHa, which harbor HPV16 but had little effect on cells that do not. Both ODNs also affected the ability of CaSki cells to form colonies in soft agar. In nude mice, treatment with either AE6, AE7, or a mixture of both led to substantially smaller tumors. AE7 was observed to inhibit E7 synthesis. The AE6 ODN probably exerts its effect by suppressing the expression of E6 as well as E7. Cell cultures and tumors treated with AE6 showed a decrease in E7 expression. In addition, an antisense ODN targeted at the retinoblastoma gene was able to reverse some of the inhibitory effect of AE6 on CaSki cells, indicating that AE6 inhibited E7 synthesis. This study further demonstrates that anti-HPV ODNs may be useful therapeutically.

INTRODUCTION

The antisense concept involves the use of short complementary ODNs[2] to interfere with the function of mRNAs. Such ODNs are usually between 12–20 bases in length. Hybridization of the antisense ODN with the target RNA by complementary base pairing provides high specificity and binding affinity resulting in the inhibition of expression of the protein product. The efficacy of antisense inhibition has been demonstrated for a variety of genes, particularly oncogenes (1–3).

HPVs are small DNA viruses with genomes of approximately 8 kb. Among the HPVs, a small subgroup including HPV16 and HPV18 are associated with cervical cancers (4). The transforming properties of these HPVs are encoded by the *E6* and *E7* genes (5–7). Both proteins may also play a role in the maintenance of the fully transformed phenotype.

Previous studies had shown that antisense ODNs to HPV16 and HPV18 E6 and E7 messages were useful in inhibiting cell growth and in affecting the transformed phenotype (8–10). However, the actual mechanism by which the anti-E6 and anti-E7 ODNs exert their effects is not well elucidated. We had shown previously that antisense ODNs complementary to *E6* and *E7* genes could specifically inhibit the proliferation of a cervical carcinoma cell line, CaSki (10). This study was carried out to further investigate the antisense inhibition of E6 and E7 in human carcinoma cells lines in culture, as well as to examine the effects of these ODNs on tumor growth in athymic nude mice.

MATERIALS AND METHODS

Synthesis of ODNs. Five different deoxyoligonucleotides were used in this study (Fig. 1). Two of these, AE6 and AE7, hybridize with sequences flanking the start codons of HPV16 E6 and E7 open reading frames, respectively. A sense ODN, HPVC, a randomized ODN, MA, and an ODN complementary to the retinoblastoma gene, ARb, were used as controls. PS ODNs of all five ODNs were custom made by Oligos Etc Inc. Before adding to the cell culture, the PS ODN was preincubated with the transfection reagent, N-[1-(2,3-Di-oleoyloxy)propyl]-N,N,N-trimethyl ammonium methylsulfate, DOTAP (Boehringer Mannhein, Mannhein, Germany), at 5 μg/ml for 10 min.

Cell Lines. Two established cervical epithelial tumor cell lines, CaSki and SiHa, containing HPV16 were used in this study. Two other cervical tumor lines, C-33A, lacking the HPV genome, and HeLa, containing HPV18, were used as control lines. CaSki, SiHa, and HeLa cells were grown in MEM supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. C-33A cells were grown in a similar medium with the addition of 0.8 mg/liter of MEM nonessential amino acids and 1 mM pyruvate.

ODN Uptake. The AE7 PS ODN was conjugated at the 5′-terminus with biotin. Cells were seeded at a density of 2×10^3 cells/well in 16-well tissue culture chamber slide vessels (Nunc, Roskilde, Denmark). After 24 h, the medium was changed, and fresh medium containing 10 μM AE7 and 5 μg/ml DOTAP was added. At 1, 4, 8, and 24 h after the addition of the ODN, the medium was removed. Cells were washed several times with PBS and fixed with 80% acetone for 10 min. The cells were then rinsed in PBS and incubated with fluorescein-conjugated strepavidin (Dako A/S, Glostrup, Denmark) for 30 min. The cells were washed again with PBS and immediately viewed by using a UV-fluorescence microscope.

Growth Assay. Cells (2×10^3) were seeded in each well of the 96-well plate and allowed to recover for 24 h before treatment with PS ODNs. Fresh medium containing 5 μg/ml DOTAP and various concentrations of PS ODNs were then introduced. Forty-eight h after the treatment, the cells were subjected to MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt) assay as described previously (10). All assays were performed in triplicates.

Anchorage-independent Growth. CaSki cells were grown in 35-mm dishes until 70–80% confluence. The cells were then treated with 20 μM of PS ODNs for 4 h, resuspended in fresh medium, and 6×10^3 viable cells were seeded on a 0.5% agarose medium and allowed to grow for 3 weeks. Colonies >100 cells were then counted. All assays were performed in duplicates, and two independent experiments were carried out.

Tumor Growth in Nude Mice. Female congenitally athymic mice, (BALB/c, *nu/nu*) between the ages of 6–8 weeks were used. Five $\times 10^6$ SiHa cells were resuspended in 0.1 ml of PBS and injected s.c. into the right hind leg of each mouse. Tumors were first visible after 10 days. Once palpable tumors were established, the mice were given injections with PS ODNs at the tumor site. At the end of the experiment, the mice were killed by N_2 euthanasia. Each tumor was then excised and weighed.

Immunological Procedures. For immunoblotting, cellular lysates from cells treated with 20 μM of PS ODNs were prepared in the lysis buffer. The lysis buffer consisted of PBS (pH 7.5) containing 0.1% SDS, 1% NP-40, 2 mM EDTA, 100 μM Na_3VO_4, 0.01% phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin. The lysates were clarified at 15,000 × *g* at 4°C for 10 min and stored at −20°C. Homogenate of tumor tissues from nude mice were also prepared in the lysis buffer and cleared by centrifugation as described. Protein concentrations were determined by using the Bio-Rad (Bio-Rad Laboratories) protein assay. Samples were fractionated on SDS-PAGE and transferred onto nitrocellulose membrane. A mouse mAb was used to detect E7 (Ciba-Corning Diagnostics Corp.), while two rabbit polyclonal antibodies were used to detect pRb (Oncogene Science) and actin (Sigma Chemical Co.). The bound antibodies were visualized by using enhanced chemiluminescence (ECL; Amersham).

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[2] The abbreviations used are: ODN, oligonucleotide; PS ODNs, phosphorothioate analogues; HPV, human papillomavirus.

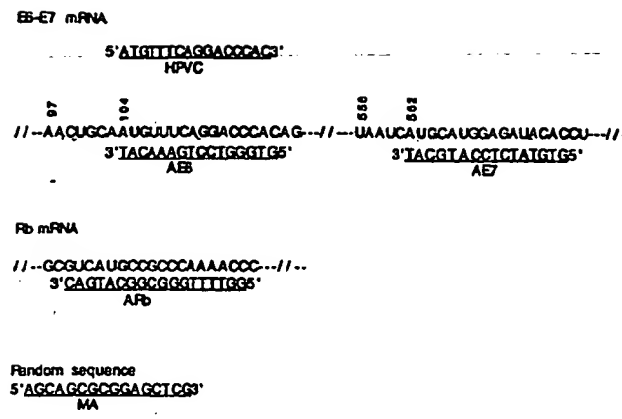

Fig. 1. Sequences of oligonucleotides used in this study and position of base pairing to the E6-E7 mRNA and Rb mRNA. HPVC correspond to the sense sequence of E6.

RESULTS

Cellular Uptake of ODNs. Uptake of the biotinylated PS ODN AE7 was monitored over a period of 24 h. Fluorescence staining could be observed in SiHa cells 1 h after incubation with the ODN and was present throughout the 24-h period (Fig. 2). A similar pattern of ODN uptake was also observed for CaSki cells (data not shown).

Growth Inhibition of CaSki and SiHa Cells. In our earlier study, it was observed that 20 μM of AE6 or AE7 could selectively inhibit the proliferation of CaSki cells (10). In this study, the effects of various concentrations and combinations of ODNs on the proliferation of CaSki and SiHa cells were examined. Cells were treated with AE6, AE7, or a mixture of both in the ratio of 1:1. Concentrations ranging from 0.1 μM to 40 μM were used.

Treatment of CaSki and SiHa cells with AE6 resulted in inhibition of cell proliferation. This inhibition ranged from 6.5 to 67.2% for CaSki cells (Fig. 3*A*) and from 11.8 to 56.1% for SiHa cells (Fig. 3*B*) when the cells were treated with concentrations varying from 5 to 40 μM. Similarly, AE7 treatment also led to inhibition of cell growth ranging from 26.0 to 60.8% for CaSki cells (Fig. 3*A*) and from 21.1 to 50.2% for SiHa cells (Fig. 3*B*) at concentrations between 1 and 40 μM. However, a combination of AE6 and AE7 was more effective, and >50% inhibition was observed at 5 μM (Table 1).

Treatment of both CaSki and SiHa cells with the control ODNs MA, HPVC, or ARb led to inhibition of <20% (Fig. 3). In addition, AE6 and AE7 had little effect on the proliferation of C-33A, which does not harbor HPV DNA, and of HeLa, which contains HPV18 (Table 2). The presence of the cationic lipid DOTAP at 5 μg/ml did not affect the proliferation of all the cells.

In another series of experiments, CaSki cells were treated for 48 h with a mixture of AE6 and ARb (2:1 ratio). The presence of ARb diminished the inhibitory effect of AE6 on the proliferation of CaSki cells (Table 3).

E7 Expression in CaSki Cells. E7 expression could be detected in CaSki cells and this expression was induced by 100 ng phorbol 12-myristate-13-acetate (TPA)/ml medium. Expression of E7 was reduced after 5 h of incubation with 20 μM AE7 (Fig. 4). The decrease in E7 expression was still evident at 24 h. However, at 24 h, the reduction in E7 protein level was less than at 5 h. A similar change in E7 expression was also observed when the cells were treated with AE6. The expression of an unrelated protein, actin, was not affected at all.

Anchorage-Independent Growth. Incubation of CaSki cells for 4 h with either AE6 or AE7 did not affect the viability of the cells (data not shown). However, cells that had been pretreated with either AE6 or AE7 formed fewer colonies on agar than did cells treated with MA or cells that were not treated (Table 4).

Tumor Growth in Nude Mice. At 5×10^6 cells, both CaSki and SiHa cells formed tumors in all mice. However, the latent period for CaSki cells was much longer than for SiHa and, hence, SiHa cells were used for all subsequent experiments. Once palpable tumors were established, the animals were divided into groups and treated with either PBS or with PS ODNs. In the initial experiment, the animals were treated for 10 days with 20 μg of MA, AE6, AE7, or with PBS.

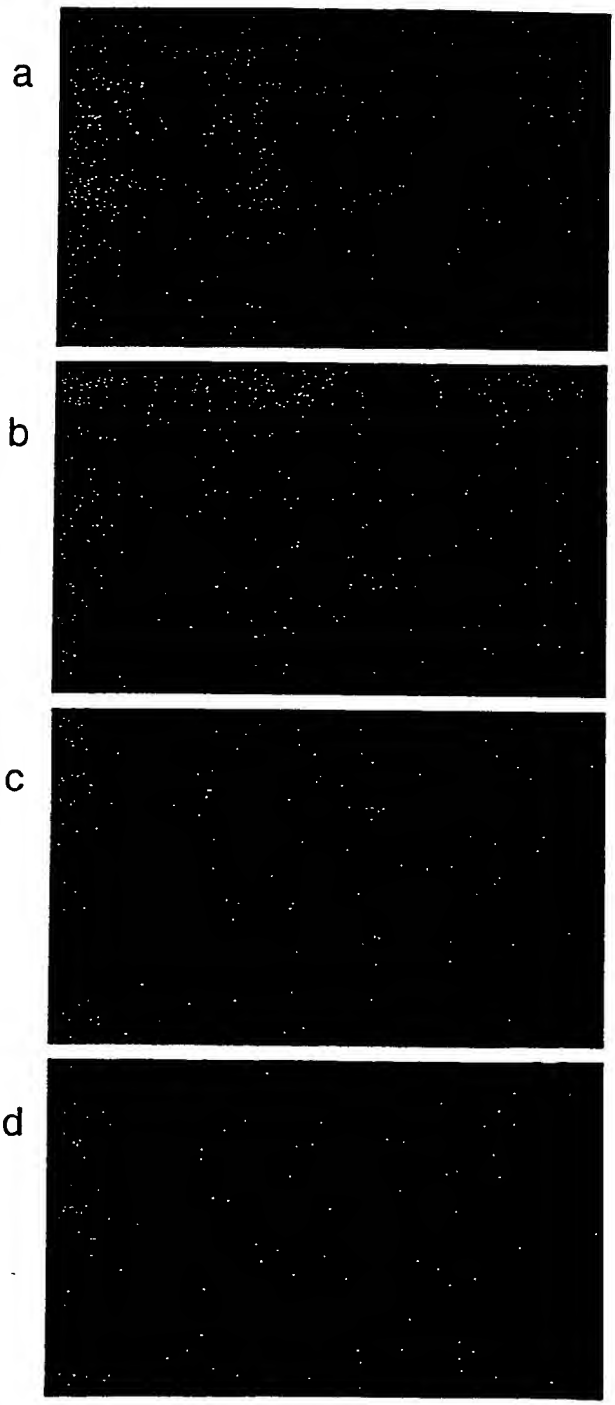

Fig. 2. Uptake of AE7 by SiHa cells at 1 (*a*), 4 (*b*), 8 (*c*), and 24 h (*d*).

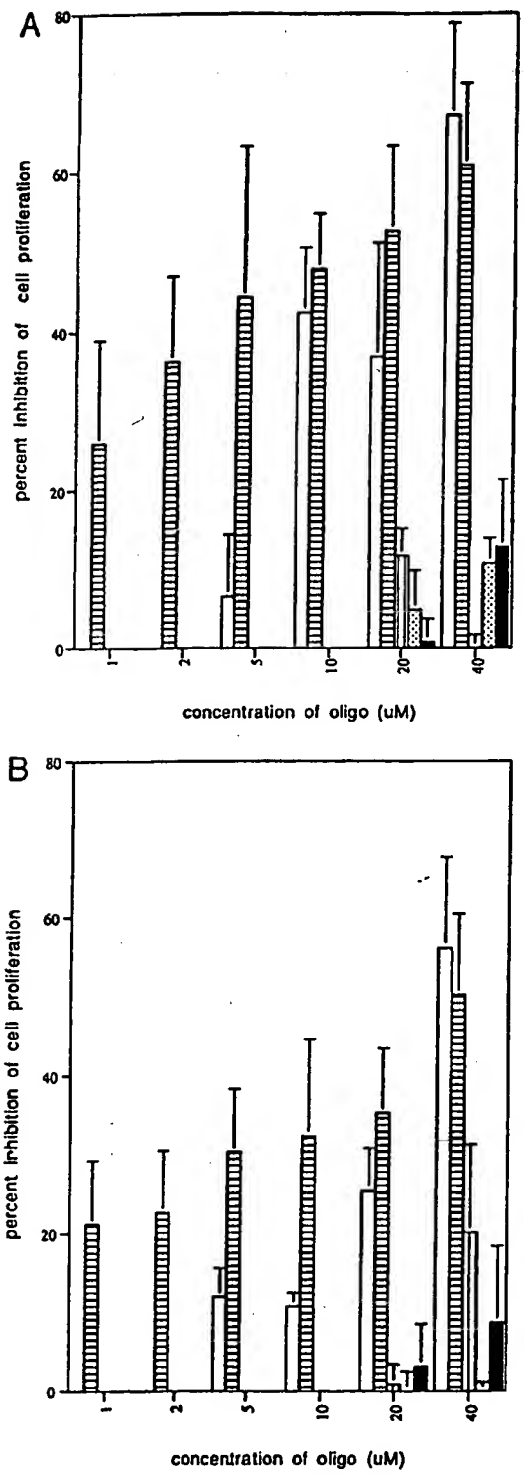

Fig. 3. Growth inhibition of CaSki cells (*A*) and SiHa cells (*B*) treated with various oligonucleotides. The cells were treated with AE6 (□), AE7 (▨), MA (▥), HPVC (▩) and ARb (■).

At the end of the treatment, animals treated with AE6 or AE7 had smaller tumors. The animals were then left untreated for 12 days before being killed. Despite the 12-day period without treatment, animals that had been previously treated with AE6 or AE7 had smaller tumors with average weight one-half that of controls (Fig. 5*A*).

In another series of experiments, all animals were treated with 30 μg of ODN daily for 15 days while the control animals were similarly treated with PBS. At the end of the treatment, animals treated with AE6 or AE7 had substantially smaller tumors (Fig. 5*B*). The average weight of these tumors was 85% lighter than those treated with PBS.

To examine if a combination of AE6 and AE7 was more effective in retarding tumor growth, the animals were treated with 10 μg of either MA, AE6, AE7, or a mixture of AE6 and AE7 in a 1:1 ratio. After 10 days of treatment, animals treated with the mixture of AE6 and AE7 had smaller tumors than those animals treated with either AE6 or AE7 (Fig. 5*C*).

Measurement of E7, pRb, and Actin in Tumors. The expressions of E7 and pRb were determined in tumors excised from the nude mice. E7 expression was reduced in tumors treated with AE6 or AE7 (Fig. 6). This decrease in E7 was accompanied by the detection of pRb. pRB was not detectable in tumors treated with PBS or MA. The expression of an unrelated protein, actin, was not affected by any of the ODNs.

DISCUSSION

In this study, we report the results of PS ODN treatments of cervical cancer cells in culture and in tumors in nude mice. The results clearly demonstrate the effectiveness of anti-HPV16 ODNs, particularly for retarding tumor growth in nude mice. Before examining the effects of the anti-HPV ODNs in nude mice, we first examined the effects on cell cultures.

In cell cultures, the uptake of the PS ODN was rapid and efficient in the presence of the cationic lipid, DOTAP. The enhancement of ODN uptake by cationic lipids has been described for a variety of cell lines (11), including CaSki (12). In this study, DOTAP was used at 5 μg/ml, and this concentration was not toxic to all the cell lines used. The ODN remained associated with the cells for up to 24 h.

Both anti-HPV ODNs, AE6 and AE7, were able to inhibit the

Table 1 *Effects of a mixture of AE6 and AE7 (1:1) on the proliferation of CaSki and SiHa cells*

Oligonucleotide concentration	% of inhibition of CaSki cell proliferation ± SD	% of inhibition of SiHa cell proliferation ± SD
0.1 μM	32.9 ± 9.1	37.5 ± 5.5
0.5 μM	40.6 ± 8.3	37.3 ± 6.4
5.0 μM	58.7 ± 11.7	50.8 ± 14.0

Table 2 *Effects of antisense oligonucleotides on the proliferation of Hela and C-33A cells*

Oligonucleotide (concentration)	% of inhibition of HeLa cell proliferation ± SD	% of inhibition of C-33A cell proliferation ± SD
AE6 (20 μM)	6.7 ± 7.1	6.6 ± 3.0
AE6 (40 μM)	13.8 ± 7.6	23.7 ± 13.3
AE7 (20 μM)	19.8 ± 8.4	0.7 ± 4.9
AE7 (40 μM)	18.8 ± 8.1	18.2 ± 3.5
MA (20 μM)	10.1 ± 8.4	ND[a]
MA (40 μM)	17.3 ± 8.1	ND
HPVC (20 μM)	16.4 ± 6.8	ND
HPVC (40 μM)	22.3 ± 7.0	ND

[a] ND, not determined.

Table 3 *Effects of antisense oligonucleotides ARb:AE6 (1:2) on the proliferation of CaSki cells*

Oligonucleotide concentration	% of inhibition of cell proliferation ± SD
30 μM	19.6 ± 2.9
40 μM	20.2 ± 8.6

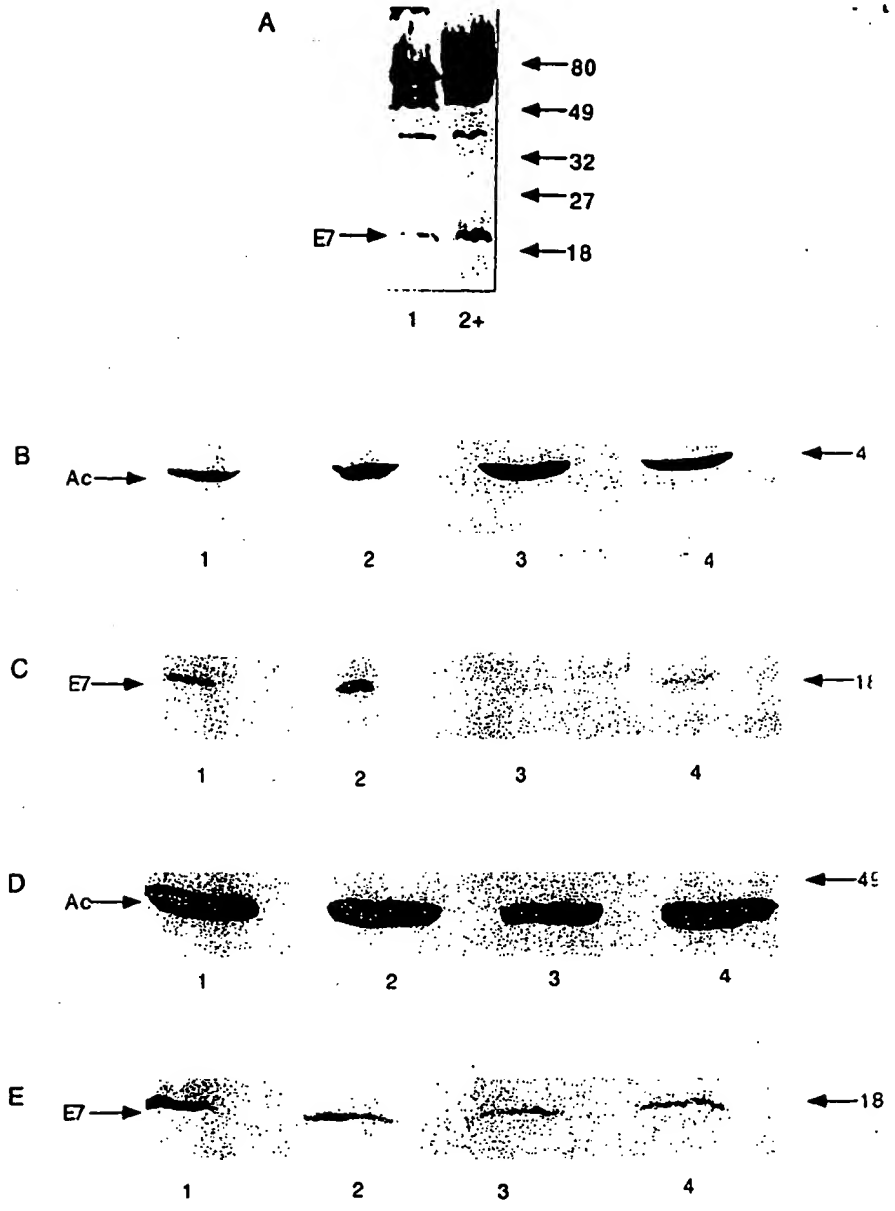

Fig. 4. Western blot analysis of E7 and actin expression in CaSki cells. *A*, detection of E7 in the absence (*Lane 1*) and presence (*Lane 2*) of 100 ng/ml TPA. *B*, actin, and *C*, E7 expression after incubation with ODNs for 5 h. *D*, actin, and *E*, E7 expression after incubation with ODNs for 24 h. *Lane 1*, lysate of untreated CaSki cells; *Lane 2*, lysate of cells treated with a control ODN, MA; *Lane 3*, lysate of cells treated with AE7; and *Lane 4*, lysate of cells treated with AE6. The positions of M_r markers are indicated on the *right* and the position of actin (*Ac*) and E7 are indicated on the *left*.

proliferation of CaSki and SiHa cells. Although Storey *et al.* (13) had reported nonspecific inhibition with similar ODNs, this study and that of Wong *et al.* (9) proved otherwise. Control ODNs did not significantly affect the growth of CaSki and SiHa cells, even at the highest concentration of 40 μM. In addition, AE6 and AE7 also decreased the ability of CaSki cells to grow on soft agar. The effects of AE6 and AE7 were specific to cells harboring HPV16, and there was little effect on the cervical line, C-33A, which has no HPV DNA, and HeLa, which contains HPV18.

In the nude mice, the anti-HPV ODNs specifically inhibited tumor growth and the control ODNs had little effect. Tumor growth was repressed by AE6 and AE7 throughout the entire period of treatment. At 12-days posttreatment, AE6-treated and AE7-treated animals still had substantially smaller tumors compared to the controls. The effect of AE6 and AE7 is probably due to a reversal of the transformed phenotype brought about by the inhibition of E6 and E7. Expression of *E6* and *E7* genes has been linked directly to the proliferative capacity of cervical cancer cells (14–16) and is also required for the maintenance of the transformed phenotype (6, 17).

Table 4 *Effect of antisense oligonucleotides on colony formation in soft agar*

Treatment	No. of colonies
Untreated	33.5
MA (20 μM)	35.5
AE6 (20 μM)	11.0
AE7 (20 μM)	9.5

The effects of AE7 on cell and tumor growth are associated with the suppression of E7 expression. In CaSki cells, the half-life of E7 is only 55 min (18). As such, it was possible to detect a decrease in E7 expression after only 5 h of incubation with AE7. However, the suppression of E7 expression was less after 24 h, and this led to only 50–60% inhibition of CaSki and SiHa cells after 48 h of treatment. E7 expression was also inhibited in tumors treated with AE7. This inhibition is specific to E7, and actin expression was not affected at all.

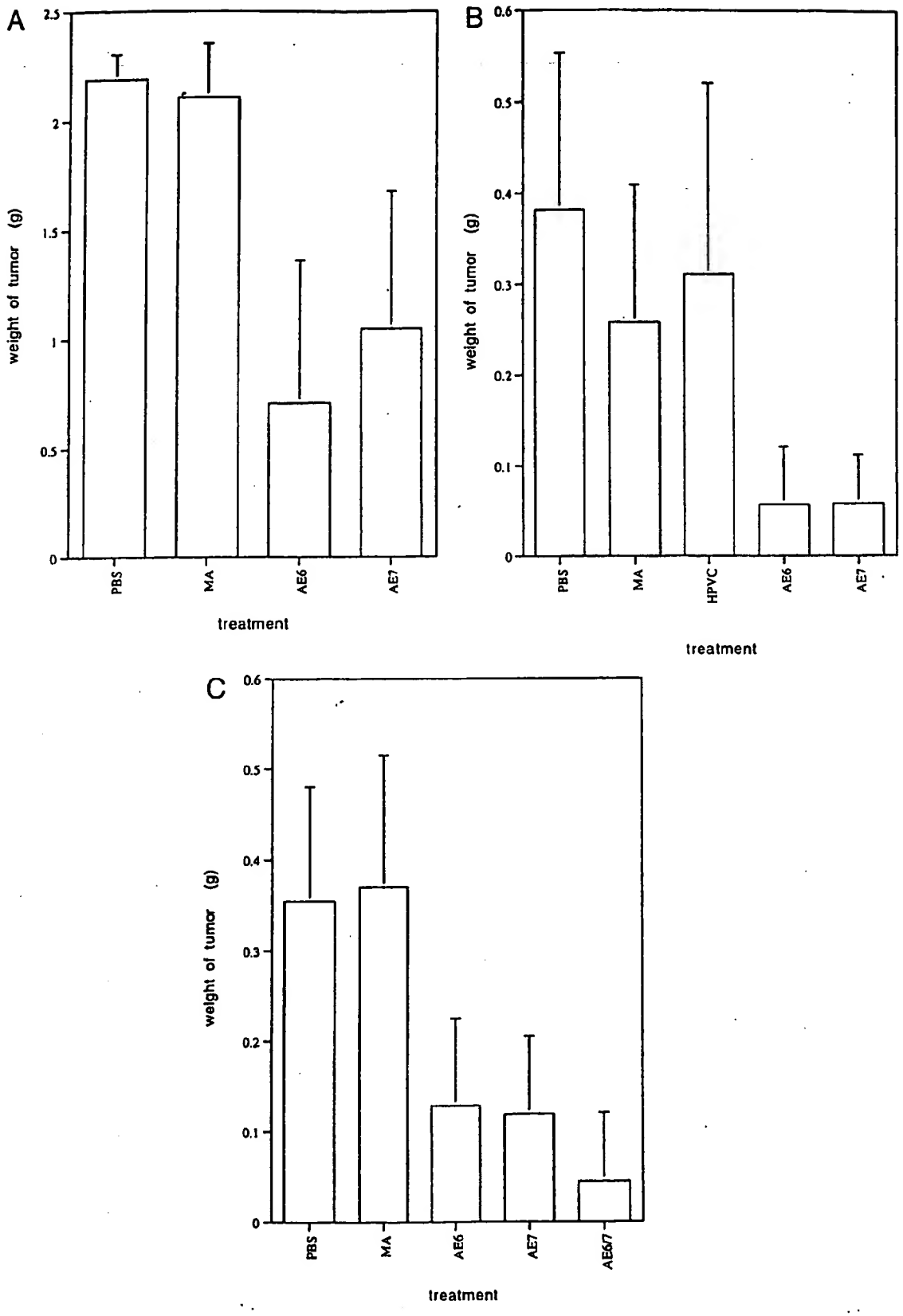

Fig. 5. Mean weight of tumor (± SD) in nude mice after s.c. injection of SiHa cells. *A*, mean weight of tumors 12 days after treatment with PBS (n = 3 mice), 20 μg of MA (n = 3 mice), 20 μg AE6 (n = 5 mice), and 20 μg AE7 (n = 5 mice). *B*, mean weight of tumors at the end of 15 days of treatment with 30 μg of oligonucleotides. Animals were treated with PBS (n = 6), MA (n = 6), HPVC (n = 6), AE6 (n = 9), or AE7 (n = 9). *C*, mean weight of tumors at the end of 10 days of treatment with 10 μg of oligonucleotides. Animals were treated with PBS (n = 4), MA (n = 7), AE6 (n = 8), AE7 (n = 5), and AE6/AE7 (n = 8).

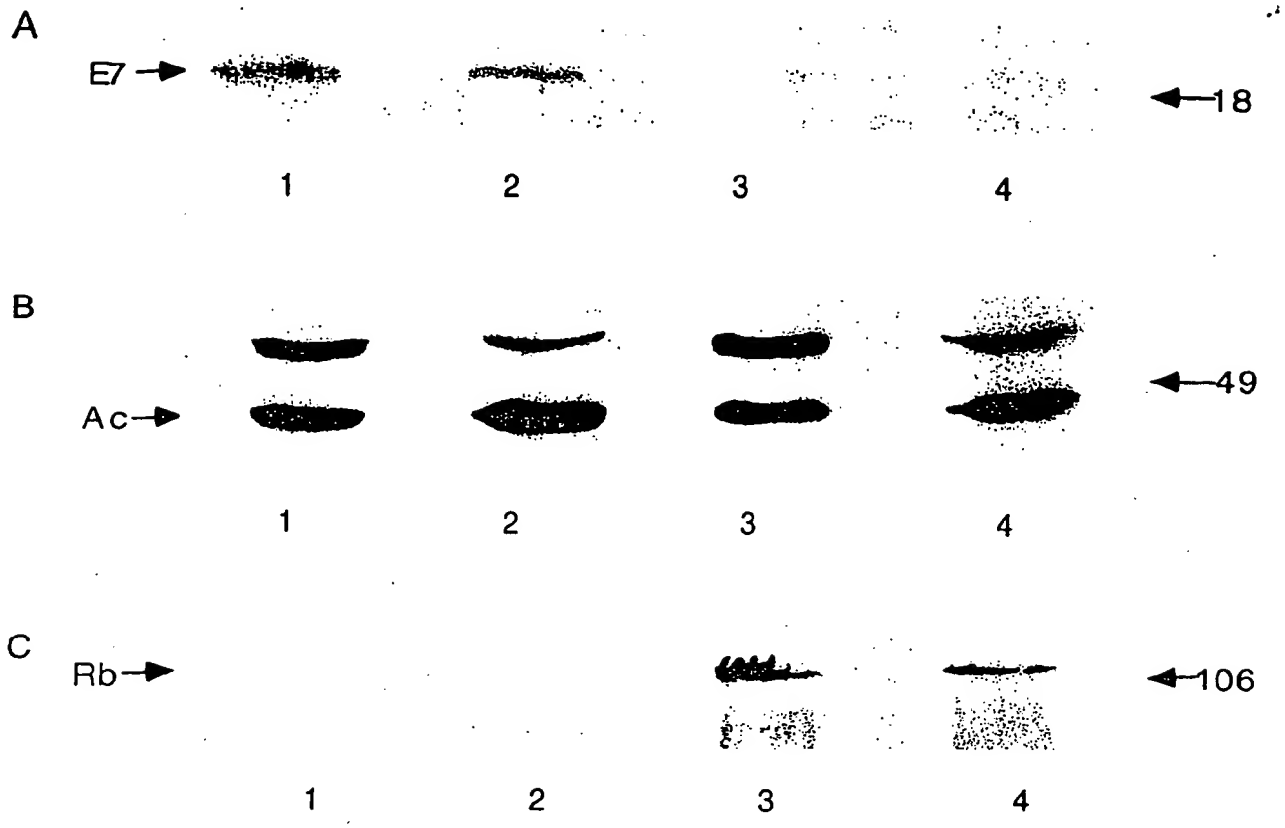

Fig. 6. Western blot analysis for E7 (*A*), actin (*B*), and pRb (*C*) proteins in tumors treated with PBS (*Lane 1*), MA (*Lane 2*), AE6 (*Lane 3*), and AE7 (*Lane 4*). The positions of *M*, markers are indicated on the *right* and the position of E7, actin (*Ac*), and pRB are indicated on the *left*.

The AE6 ODN probably exerts its effect through the suppression of E6 as well as the expression of E7. In cervical cancers and cervical cell lines containing HPV, mRNAs encoding E6 and E7 proteins are transcribed from the same promoter in the form of a bicistronic transcript (19, 20). *In vivo*, the bicistronic transcript is also spliced to produce two shorter transcripts E6*I-E7 and E6*II-E7 (21). It has been proposed that it is a function of these spliced transcripts to stimulate E7 translation (19, 20). Because all three transcripts have the same 5′ ODN sequence, one would assume that AE6 could affect all three because regions at the 5′ end of the mRNA are most sensitive to antisense inhibition of translation (22–24).

Our data does indeed indicate that AE6 affects all three transcripts, thereby, inhibiting E7 translation. *In vitro*, inhibition of the full-length bicistronic E6-E7 transcript by AE6 results in the suppression of both E6 and E7 expression. This was achieved by the specific hybridization of AE6 with the E6 sequence and not through nonspecific interaction of AE6 with the E7 sequence (10). E7 is known to bind the product of the retinoblastoma susceptibility gene, *pRb* (25), thereby, preventing pRb from regulating cell growth. Inhibition of E7 will, thus, allow pRb to perform its function of regulating cell growth. For CaSki cells, the inhibition of cell proliferation by AE6 can be reversed by inhibiting pRb expression by using the ODN ARb, which has been shown to be effective in inhibiting the synthesis of pRb in human embryonic lung fibroblasts (26). This reversal suggests that part of the effects of AE6 is achieved via the inhibition of E7 expression. In addition, the expression of E7 in the tumors in nude mice probably caused the Rb protein to be unstable and, hence, Rb could only be detected in tumors treated with AE6 or AE7 because both ODNs suppress E7 expression. The reduction of E7 expression in tumors and cells treated with AE6 indicates that AE6 probably also inhibits E7 expression from the two shorter transcripts, E6*I-E7 and E6*II-E7, because *in vivo*, these two transcripts account for >80% of the major E6-E7 transcripts (21).

Antisense inhibition of E6 and E7 has been shown to be useful in inhibiting the growth of cervical cells harboring HPV16 or HPV18 DNA (8–10). This study also shows that anti-E6, as well as anti-E7 ODNs, are useful in impeding tumor growth in nude mice. A combination of AE6 and AE7 was more effective than each of the ODNs individually. These observations, together with the specificity of the antisense inhibition, augurs well for the therapeutic potential of antisense ODNs in HPV-related cervical cancers.

REFERENCES

1. Stein, C. A., and Cheng, Y-C. Antisense oligonucleotides as therapeutic agents—Is the bullet really magical? Science (Washington DC), *261:* 1004–1012, 1993.
2. Crooke, S. T. Therapeutic applications of oligonucleotides. Biotechnology, *10:* 882–886, 1992.
3. Neckers, L., Whitesell, L., Rosolen, A., and Geselowitz, D. A. Antisense inhibition of oncogene expression. Crit. Rev. Oncog., *3:* 175–231, 1992.
4. DeVilliers, E. M. Heterogeneity of the human papillomavirus group. J. Virol., *63:* 4898–4903, 1989.
5. Hawley-Nelson, P., Vousden, K. H., Hubbert, N. L., Lowy, D. R., and Schiller, J. T. HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. EMBO J., *8:* 3905–3910, 1989.
6. Münger, K., Phelps, W. C., Bubb, V., Howley, P. M., and Schlegel, R. The *E6* and *E7* genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J. Virol., *63:* 4417–4421, 1989.
7. Hudson, J. B., Bedell, M. A., McCance, D. J., and Laimins, L. A. Immortalization and altered differentiation of human keratinocytes *in vitro* by the E6 and E7 open reading frames of human papillomavirus type 18. J. Virol., *64:* 519–526, 1990.
8. Steele, C., Cowsert, L. M., and Shillitoe, E. J. Effects of human papillomavirus type 18-specific antisense oligonucleotides on the transformed phenotype of human carcinoma cell lines. Cancer Res., *53:* 2330–2337, 1993.

9. Wong, Y. F., Chung, T. K. H., Cheung, T. H., Lam, S. K., and Chang, A. M. Z. Effects of human papillomavirus type-specific antisense oligonucleotides on cervical cancer cells containing papillomavirus type 16. Med. Sci. Res., *22:* 511–513, 1994.
10. Tan, T. M. C., Gloss, B., Bernard, H-U., and Ting, R. C. Y. Mechanism of translation of the bicistronic mRNA encoding human papillomavirus type 16 *E6-E7* genes. J. Gen. Virol., *75:* 2663–2670, 1994.
11. Capaccioli, S., Pasquale, G. D., Mini, E., Mazzei, T., and Quattrone, A. Cationic lipids improve antisense oligonucleotide uptake and prevent degradation in cultured cells and in human serum. Biochem. Biophys. Res. Commun., *197:* 818–825, 1993.
12. Lappalainen, K., Urtti, A., Jääskeläinen, I., Syrjänen, K., and Syrjänen, S. Cationic liposomes mediated delivery of antisense oligonucleotides targeted to HPV16 E7 mRNA in CaSki cells. Antiviral Res., *23:* 119–130, 1994.
13. Storey, A., Oates, D., Banks, L., Crawford, L., and Crook, T. Anti-sense phosphorothioate oligonucleotides have both specific and non-specific effects on cells containing human papillomavirus type 16. Nucleic Acids Res., *19:*4109–4114, 1991.
14. von Kenbel Doeberitz, M., Oltersdorf, T., Schwarz, E., and Gissman, L. Correlation of modified human papillomavirus early gene expression with altered cell growth in C4-1 cervical cancer cells. Cancer Res., *48:* 3780–3786, 1988.
15. von Kenbel Doeberitz, M., Gissman, L., and zur Hausen, H. Growth-regulating functions of human papillomavirus early gene products in cervical cancer cells acting dominant over enhanced epidermal growth factor receptor expression. Cancer Res., *50:* 3730–3736, 1990.
16. Watanabe, S., Kanda, T., and Yoshiike, K. Growth dependence of human papillomavirus 16 DNA-positive cervical cancer cell lines and human papillomavirus 16-transformed human and rat cells on the viral oncoproteins. Jpn. J. Cancer Res., *84:* 1043–1049, 1993.
17. Crook, T., Morgenstern, J. P., Crawford, L., and Banks, L. Continued expression of HPV16 E7 protein is required for maintenance of the transformed phenotype of cells co-transformed by HPV-16 plus EJ-ras. EMBO J., *8:* 513–519, 1989.
18. Smotkin, D., and Wettstein, F. O. The major human papillomavirus protein in cervical cancers is a cytoplasmic phosphoprotein. J. Virol., *61:* 1686–1689, 1987.
19. Schneider-Gädicke, A., and Schwarz, E. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. EMBO J., *5:* 2285–2292, 1986.
20. Smotkin, D., and Wettstein, F. O. Transcription of human papillomavirus type 16 early genes in a cervical cancer and a cancer-derived cell line and identification of the E7 protein. Proc. Natl. Acad. Sci. USA, *83:* 4680–4684, 1986.
21. Shirasawa, H., Tanzawa, H., Matsunaga, T., and Simizu, B. Quantitative detection of spliced E6-E7 transcripts of human papillomavirus type 16 in cervical premalignant lesions. Virology, *184:* 795–798, 1991.
22. Sankar, S., Cheah, K. C., and Porter, A. G. Antisense oligonucleotide inhibition of encephalomyocarditis virus RNA translation. Eur. J. Biochem., *184:* 39–45, 1989.
23. Maher, L. J., III, and Dolnick, B. J. Specific hybridization arrest of dihydrofolate reductase mRNA in *vitro* using antisense RNA or antisense oligonucleotides. Arch. Biochem. Biophys., *253:* 214–220, 1987.
24. Gupta, K. C. Antisense oligonucleotides provide insight into mechanism of translation initiation of two sendai virus mRNAs. J. Biol. Chem., *262:* 7492–7496, 1987.
25. Dyson, N., Howley, P., Münger, K., and Harlow, E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science (Washington DC), *243:* 934–937, 1989.
26. Strauss, M., Hering, S., Lieber, A., Herrmann, G., Griffin, B. E., and Arnold, W. Stimulation of cell division and fibroblast focus formation by antisense repression of retinoblastoma protein synthesis. Oncogene, *7:* 769–773, 1992.